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Biological Aspects in Renal Cell Carcinoma

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1. Introduction

A growing understanding of the underlying molecular biology of renal cell carcinoma (RCC) has identified several pathways pertinent to its pathophysiology. Cellular hypoxia and metabolic stress have been observed in many cancer types. Hypoxia-induced factor (HIF) is considered a central regulator of oxygen homeostasis. The HIF transcription factor complex has been demonstrated to transcriptionally induce the expression of genes involved in angiogenesis, anaerobic glucose metabolism, cell motility and metastasis, growth and survival, apoptosis, and telomere maintenance. Notable genes induced by HIF that are involved in angiogenesis include vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF), as well as other proangiogenic factors, such as angiopoietin-4 (Ang4). These factors promote the proliferation, migration, and maturation of endothelial cells and pericytes supporting the recruitment of vessels or neoangiogenesis necessary to restore blood supply to an ischemic region. In the case of RCC, this process leads to the rampant, disorganised proliferation of vessels in this highly vascular tumour type. Additional factors include proteins involved in promoting the cellular switch to anaerobic glycolysis, such as the glucose transporter Glut1; enzymes of glucose metabolism, such as hexokinase (HK) and lactate dehydrogenase (LDH), the antigen carbonic anhydrase IX (CAIX, also called G250) and the lactate transporter MCT-4. This hypoxic repertoire of gene upregulation likely contributes to the highly glycolytic phenotype of RCC, even in the presence of abundant oxygen with which to perform oxidative phosphorylation for energy generation. Like other processes that integrate endothelial cell vascular network expansion, tumour angiogenesis is dependent on secreted VEGF to promote existing vessel in growth into the tumour and the expansion of vascular networks by neovascularisation. Inappropriate activation of the hypoxia response pathway is a major mechanism of VEGF transcriptional regulation in RCC. A variety of mechanisms account for the increase in VEGF, with activation of the hypoxic response pathway via the transcription factors HIF1-α and HIF2-α as the classic mechanism of induction.

RCC presents a unique clinical setting, in which a tumour type nearly universally usurps a proangiogenic cellular homeostatic mechanism.

Knowledge of the genetic basis of RCC has important implications for diagnosis and management of this disease. Study of the genes for RCC has revealed that kidney cancer is

fundamentally a metabolic disorder. The seven RCC genes (*VHL*, *MET*, *BHD*, *TSC1*, *TSC2*, *fumarate hydratase* (*FH*) and *succinate dehydrogenase* (*SDH*)) represent disorders of energy, nutrient, iron and oxygen sensing.

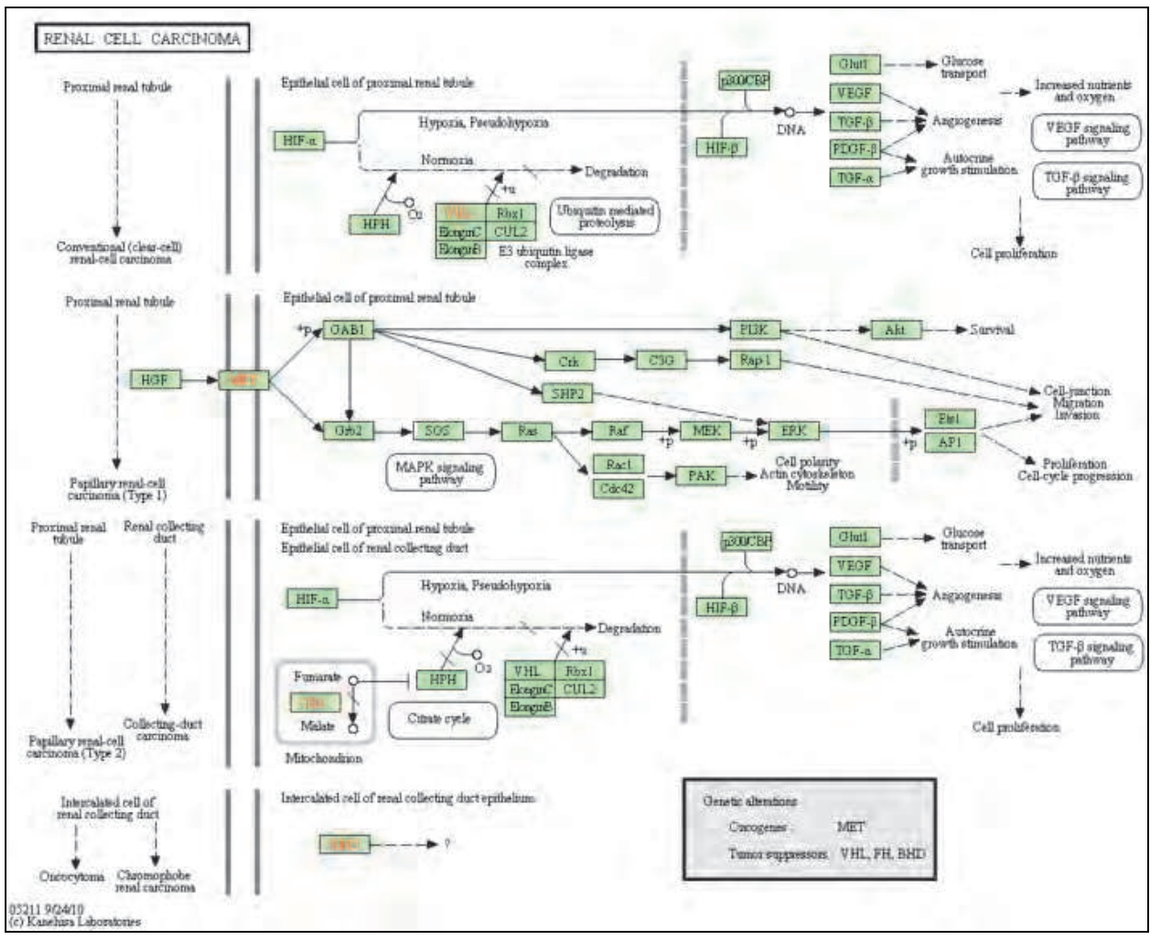


Fig. 1. Renal Cell Carcinoma Pathway (Kanehisa et al., 2010). Renal cell cancer (RCC) accounts for ~3% of human malignancies and its incidence appears to be rising. Although most cases of RCC seem to occur sporadically, an inherited predisposition to renal cancer accounts for 1-4% of cases. RCC is not a single disease, it has several morphological subtypes. Conventional RCC (clear cell RCC) accounts for ~80% of cases, followed by papillary RCC (10-15%), chromophobe RCC (5%), and collecting duct RCC (<1%). Genes potentially involved in sporadic neoplasms of each particular type are VHL, MET, BHD, and FH respectively. In the absence of VHL, hypoxia-inducible factor alpha (HIF- α) accumulates, leading to production of several growth factors, including vascular endothelial growth factor and platelet-derived growth factor. Activated MET mediates a number of biological effects including motility, invasion of extracellular matrix, cellular transformation, prevention of apoptosis and metastasis formation. Loss of functional FH leads to accumulation of fumarate in the cell, triggering inhibition of HPH and preventing targeted pVHL-mediated degradation of HIF- α. BHD mutations cause the Birt-Hogg-Dube syndrome and its associated chromophobe, hybrid oncocytic, and conventional (clear cell) RCC.

Targeting the basic metabolic alterations in RCC has the potential to provide a more durable and effective approach to therapy.

2. Overview of hypoxia-inducible signalling

Over the last decade, major advances have been made in deciphering the molecular mechanisms that allow cells to respond and adapt to low PO2. As key mediators in cellular oxygen homeostasis, hypoxia-inducible factor-1 and -2 (HIF-1 and HIF-2) facilitate both oxygen delivery and adaptation to oxygen deprivation by regulating the expression of gene products that are involved in cellular energy metabolism and glucose transport, angiogenesis, erythropoiesis and iron metabolism, pH regulation, apoptosis, and cell proliferation, as well as cell-cell and cell-matrix interactions (Schofield et al., 2004). Examples of classic HIF target genes are phosphoglycerate kinase-1 (PGK), glucose transporter-1 (GLUT1), vascular endothelial growth factor (VEGF), and erythropoietin (EPO).

HIF-1 and HIF-2 (collectively referred to here as HIF) are members of the Per-ARNT-Sim (PAS) family of heterodimeric basic helix-loop-helix (bHLH) transcription factors and consist of an oxygen-sensitive α-subunit and a constitutively expressed β-unit, also known as the aryl hydrocarbon receptor nuclear translocator (ARNT) or simply HIF-β.

Direct transcriptional regulation occurs through the binding of HIF heterodimers to hypoxia-response elements (HREs), which are present in the regulatory regions of hypoxia-sensitive genes. With regard to their ability to transcriptionally regulate specific hypoxia-responsive genes, HIF-1 and HIF-2 have distinct functions and only partially overlap. For example, glycolytic genes appear to be predominantly regulated by HIF-1 (Hu et al., 2003), whereas HIF-2 has been suggested as the main regulator of hypoxic VEGF and EPO induction in tissues that express both HIF-1 and HIF-2 (Rankin et al., 2005).

In addition to heterodimerisation with HIF-β resulting in the formation of a bHLH transcription factor that mediates the canonical hypoxia response, HIF-α subunits also regulate biological processes through direct protein-protein interaction with other factors. These include, among others, the tumour suppressor protein p53 and the c-Myc proto-oncogene (Koshiji et al., 2004). A more recent example is the ability of HIF-1α to biochemically associate with the intracellular domain of Notch (Notch ICD), thereby increasing Notch signalling through upregulation of Notch target genes such as Hey and Hes (Gustafsson et al., 2005). The observation that HIF-1α modulates Notch signalling through a direct protein-protein interaction underscores the importance of HIF-α as a regulator of important intracellular pathways, independent of its role in HRE-mediated transcription.

HIF activation is dependent on the stabilisation of the oxygen-sensitive α-subunit and its subsequent translocation to the nucleus, where it dimerises with HIF-β and recruits transcriptional cofactors such as CBP and p300 (Schofield et al., 2004). Normally, under conditions of adequate oxygen supply, hydroxylated HIF-α binds to the von Hippel-Lindau tumour suppressor protein (pVHL), which is part of an E3-ubiquitin ligase complex that targets HIF-α for proteasomal degradation. The pVHL/HIF-α interaction is highly conserved between species and requires iron- and oxygen-dependent hydroxylation of

specific proline residues within the oxygen-dependent degradation domain (ODD) of HIF-α. Prolyl-hydroxylation by prolyl-4-hydroxylases and binding to pVHL are absolutely required for the execution of HIF proteolysis under normoxia. During hypoxia, prolyl-hydroxylases are inactive, and HIF-α degradation is inhibited. Three major mammalian HIF prolyl-hydroxylases have been identified, all of which are expressed in renal epithelial cells (Soilleux et al., 2005).

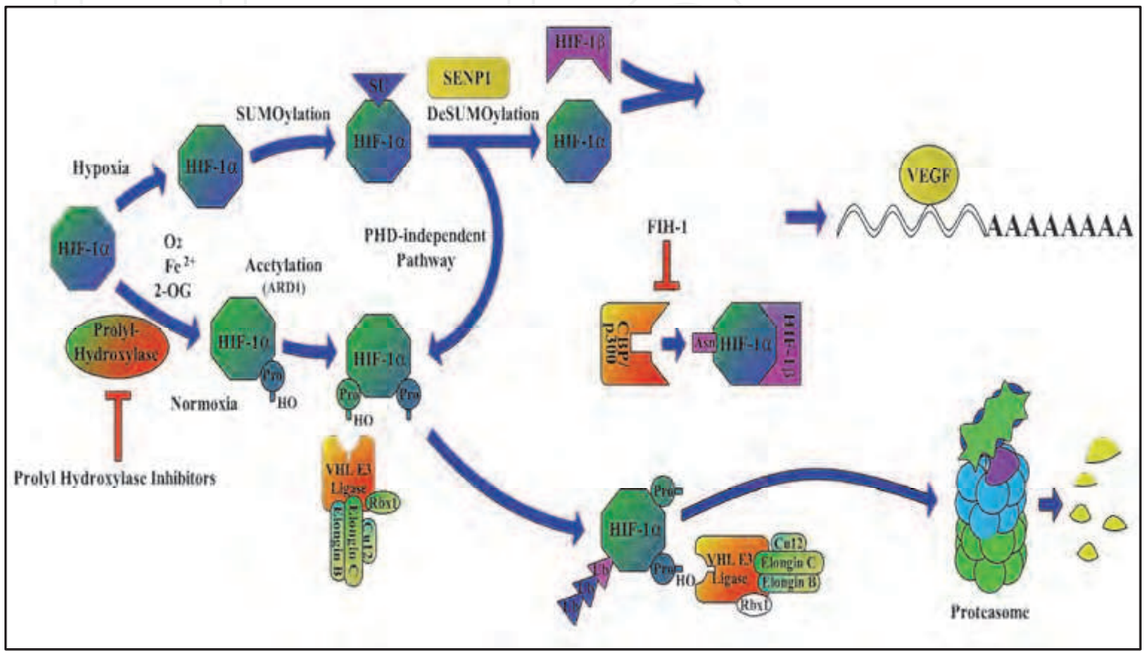


Fig. 2. Regulatory pathway of HIF. HIF-α is hydroxylated in the oxygen dependent destruction domain and at an asparagine residue in the C terminal transactivation domain. Prolyl hydroxylation by PHD is required for binding to the pVHL-E3-ubiquitin ligase complex, whereas asparaginyl hydroxylation prevents the interaction of HIF-α with CBP/p300 transcriptional co-activator. Upon binding to the E3-ligase complex, HIF-α is polyubiquitinated and then degraded by the proteasome. Acetylation of HIF-α by ARD1 enhances binding to pVHL and subsequent ubiquitination. During hypoxia when prolyl-hydroxylases are inactive, HIF-α is stabilized and translocates to the nucleus, where it is SUMOylated by SUMO conjugases. Failure to deSUMOylate HIF-α targets HIF-α for VHL/proteasome-dependent degradation by providing an alternate signal (prolyl hydroxylation-independent) for pVHL binding. DeSUMOylated HIF-α escapes degradation, heterodimerizes with HIF-1β, binds to the hypoxia response elements (consensus binding site), and increases transcription of HIF target genes such as VEGF. Oxygen-dependent asparagine hydroxylation of HIF-α by factor-inhibiting HIF-1 (FIH 1) blocks recruitment of the CBP/p300 co-factor to the HIF transcriptional complex.

A second hypoxic switch operates in the COOH-terminal transactivation domain of HIF-α upon the hydroxylation of a specific asparagine residue. During hypoxia, asparagine hydroxylation is blocked, and CBP/p300 recruitment is facilitated, enabling increased levels of transcription. Factor-inhibiting-HIF (FIH) hydroxylates the asparagine residue. FIH is expressed in renal tubular epithelial cells and glomeruli (Soilleux et al., 2005).

In addition to hypoxic activation, a nonhypoxic increase in HIF transcriptional activity has been shown to be mediated by nitric oxide and TNF-α (Sandau et al., 2001), interleukin 1 (Stiehl et al., 2002), angiotensin II (Richard et al., 2000), and a variety of growth factors, including epidermal growth factor, insulin, and insulin-like growth factors (Jiang et al., 2001; Stiehl et al., 2002; Treins et al., 2002). Nitric oxide, ROS, and certain oncogenes such as v-Src and activated Ras have been shown to inhibit HIF prolyl-hydroxylation (Kaelin 2005). In contrast, HIF activation induced through the phosphoinositide 3-kinase/Akt-1/mammalian target of rapamycin pathway appears to be mediated through increased HIF-α protein translation (Fukuda et al., 2002). Thus, HIF activation is likely to occur in a variety of different renal disease settings even in the absence of significant hypoxia.

Whereas HIF-1α is ubiquitously expressed, HIF-2α expression is more restricted. HIF-2α has been found in hepatocytes, cardiomyocytes, glial cells, type II pneumocytes, and endothelial cells (Wiesener et al., 2003).

The list of HIF-regulated genes (either directly or indirectly regulated by HIF) has grown rapidly. HIF is involved in the regulation of a multitude of biological processes that are relevant to kidney function under physiological and pathological conditions.

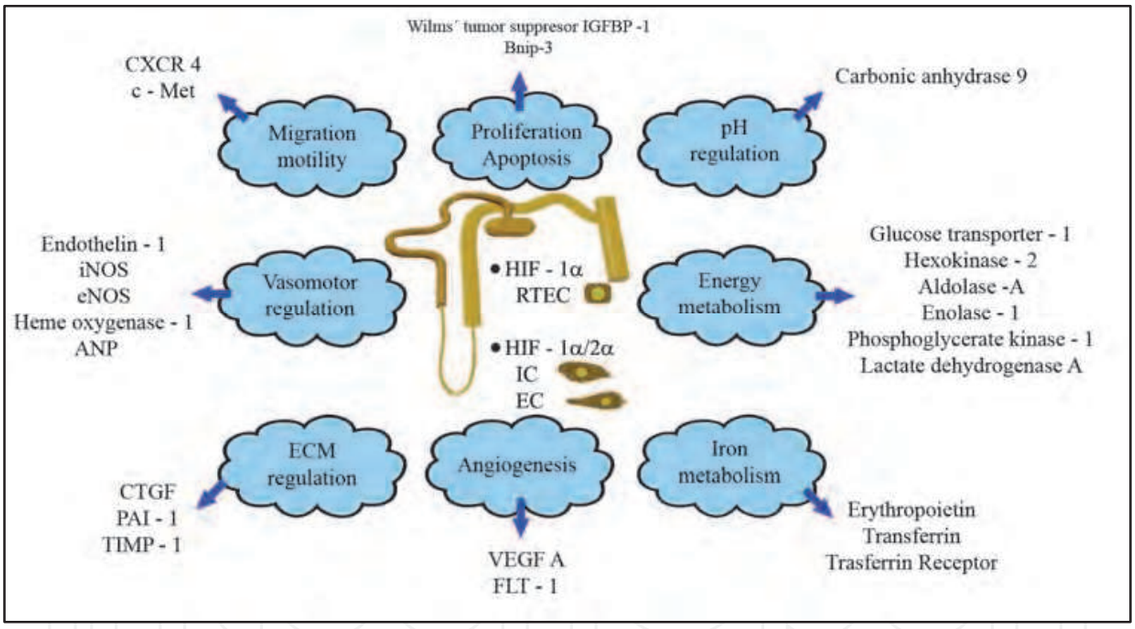


Fig. 3. Overview of selected hypoxia/HIF-regulated biological processes that have been shown or have been proposed to play important roles in the pathogenesis of renal cancer. RTEC (renal tubular epithelial cell), IC (intersticial cell), EC (endothelial cell).

3. Renal injury in cancer patients: Hypoxia and Inflammation

Whereas acutely injured kidneys appear to benefit from the protective effects of HIF-regulated biological processes, chronic hypoxia, mediated in part through HIF-1, can contribute to increased extracellular matrix production and the epithelial-to-mesenchymal transition (EMT), thereby potentially promoting renal fibrosis and the progression of renal disease. HIF may impact the pathogenesis of tubulointerstitial disease through the

regulation of inflammatory responses. Microenvironmental changes, such as hypoxia, strongly impact inflammatory cell recruitment (Kong et al., 2004) and function (Cramer et al., 2003). HIF-1 is essential for myeloid cell-mediated inflammation mainly through its effects on cellular ATP generation. Inactivation of HIF-1 results in a profound impairment of myeloid cell aggregation, motility, and invasiveness, whereas forced expression of HIF-1 has the opposite effect (Kong et al., 2004). Alterations in HIF signalling in inflammatory cells may also play a significant role in renal inflammation, subsequent fibrosis and, thus, the progression of chronic renal disease.

Patients with neoplasia are subject to a variety of different types of renal, fluid and electrolyte disorders, either from direct effects such as urinary tract obstruction or infiltration or from indirect effects such as hyperkalaemia. Glomerulonephritis and nephrotic syndrome are due to immunologic reactions associated with neoplasia.

Just as hypoxia can induce inflammation, inflamed lesions often become severely hypoxic. Because of the steep oxygen gradient between the anaerobic intestinal lumen and the metabolically active lamina propria mucosae, intestinal epithelial cells are normally hypoxic (Karhausen et al., 2004).

Contributors to tissue hypoxia during inflammation include an increase in the metabolic demands of cells and a reduction in metabolic substrates caused by thrombosis, trauma, compression (interstitial hypertension), or atelectasis (airway plugging). Moreover, multiplication of intracellular pathogens can deprive infected cells of oxygen (Kempf et al., 2005). In the case of inflamed tissue, hypoxia is not a bystander; rather, it can influence the environment of the tissue, particularly by regulating oxygen-dependent gene expression.

Activation of the prolyl hydroxylase (PHDs)–HIF pathway promotes the resolution of mucosal inflammation in mice (Colgan et al., 2010). Hypoxia-induced changes in gene expression by epithelial cells help to promote mucosal barrier function (e.g., through the activation of intestinal trefoil factor) (Furuta et al., 2001) or to increase the production by the epithelium of anti-inflammatory signalling molecules such as adenosine (Eltzschig 2009). These adaptive responses to hypoxia are activated during mucosal inflammation and promote the resolution of inflammatory bowel disease (Karhausen et al., 2004; Robinson et al., 2008; Eckle et al., 2008) and acute lung injury (Rosenberg et al., 2009; Reutershan et al., 2009; Schingnitz et al., 2010). Several studies have shown that hypoxia enhances the enzymatic conversion of precursor nucleotides, such as ATP, adenosine diphosphate, or AMP, to adenosine (Eltzschig et al., 2003), thereby elevating extracellular levels of adenosine, an anti-inflammatory signalling molecule involved in restraining innate immune responses.

HIF stimulates the production of extracellular adenosine and suppresses both its uptake into the intracellular compartment and its intracellular metabolism (Morote-García et al., 2008). HIF also enhances adenosine receptor signalling by increasing the expression on the cell surface of adenosine receptors (Eckle et al., 2008), an effect that attenuates immune responses, vascular fluid leakage, and neutrophil accumulation in the presence of myocardial, renal, hepatic, or intestinal ischemia or acute lung injury.

Concentrations of oxygen in solid tumors, as compared with those in normal tissues, are frequently lower (Semenza 2003). Solid tumours contain increased levels of HIF-1α and HIF-2α. Hypoxia in a solid tumour stabilises HIF through hypoxia-dependent inhibition of PHDs. Similarly, the activation of oncogenes, or the loss of function of tumour-suppressor

genes, results in the stabilisation of HIF, as happens in the case of the VHL tumour-suppressor gene. Hypoxia and inflammation meet at several points in the setting of cancer. In tumour cells oncogenes, inflammatory signals (mediated in part through Toll-like receptors [TLRs]), and hypoxia-activated nuclear factor κB (NF-κB) and hypoxia-inducible factor (HIF) 1α (which activate one another). These factors induce a gene program that recruits and activates leukocytes (through the release of chemokines and cytokines), stimulates angiogenesis and the formation of an abnormal vasculature and endothelium (through release of angiogenic signals), and increases tumour-cell invasion, metastasis, epithelial-to-mesenchymal transition (EMT), survival, proliferation, and metabolic reprogramming. In leukocytes, hypoxia also activates NF-κB and HIF-1α; endogenous ligands, released from necrotic cancer cells, activate TLRs upstream of NF-κB and HIF-1α, and HIF-1α up-regulates TLR expression. The resultant gene-expression profile leads to the production of cytokines and angiogenic signals and skews their polarisation phenotype. Tumour vessels with two PHDs-domain 2 (PHD2) alleles have an abnormal endothelium, are hypo-perfused, and cause tumour hypoxia, which fuels tumour-cell invasiveness and metastasis. In contrast, tumour vessels lacking one PHD2 allele have increased HIF-2α levels, which results in an up-regulation of factors that counteract the development of tumour endothelial abnormalities; this, in turn, results in improved tumour-vessel perfusion and oxygenation and, secondarily, reduced metastasis.

Experimental evidence indicates that inhibition of HIF within the inflamed tumour core attenuates the growth and vascularisation of tumours and enhances the sensitivity of tumours to radiation (Semenza et al., 2010). In contrast, inhibition of PHD2 and stabilisation of HIF within the tumour vasculature may play an important role in tumour therapy, if the means can be found to selectively direct inhibitors of PHD to the tumour vasculature and inhibitors of HIF to the hypoxic core.

4. Hypoxia inducible factor and renal cancer

The most common form of kidney cancer is renal cell cancer of the clear cell type (CC-RCC). A molecular hallmark of sporadic CC-RCC and hereditary CC-RCC associated with the von Hippel-Lindau familial tumour syndrome are mutations in the VHL tumour suppressor pVHL. Loss of pVHL function results in oxygen-independent HIF-α stabilisation, increased HIF transcriptional activity, and constitutive upregulation of HIF target genes. While patients with sporadic CC-RCC are characterised by somatic inactivation of both VHL gene copies in renal epithelial cells, patients with the VHL tumour syndrome transmit germ line mutations of the VHL gene. Although the highly vascular nature of VHL-deficient tumours is easily explained by increased VEGF production as a result of increased HIF transcriptional activity, VHL-associated renal carcinogenesis is more difficult to understand and most likely requires multiple other genetic events beyond the loss of pVHL function. In addition to regulating the degradation of HIF-α-subunits, pVHL has been shown to have additional biological functions, which may or may not be critical for renal tumourigenesis (Haase 2005).

Aside from a regulatory role in tumour angiogenesis, HIF plays a key role in the regulation of factors that are important for the development and invasiveness of CC-RCC. These include, among others, TGF-α, a potent renal epithelial mitogen, cell cycle regulator cyclin D1 (CCND1), and chemokine receptor CXCR4 (Bindra et al., 2002; Raval et al., 2005; Smith et

al., 2005; Staller et al., 2003; Wykoff et al., 2004; Zatyka et al., 2002). With regard to the individual contribution of HIF-1 and HIF-2 to renal tumour development, a substantial number of VHL-defective CC-RCC cell lines do not express HIF-1α but do express HIF-2α (Maxwell et al., 1999). This is in contrast to normal, nontransformed renal epithelial cells, in which HIF-2α is not detectable during ischemia (Rosenberger et al., 2002).

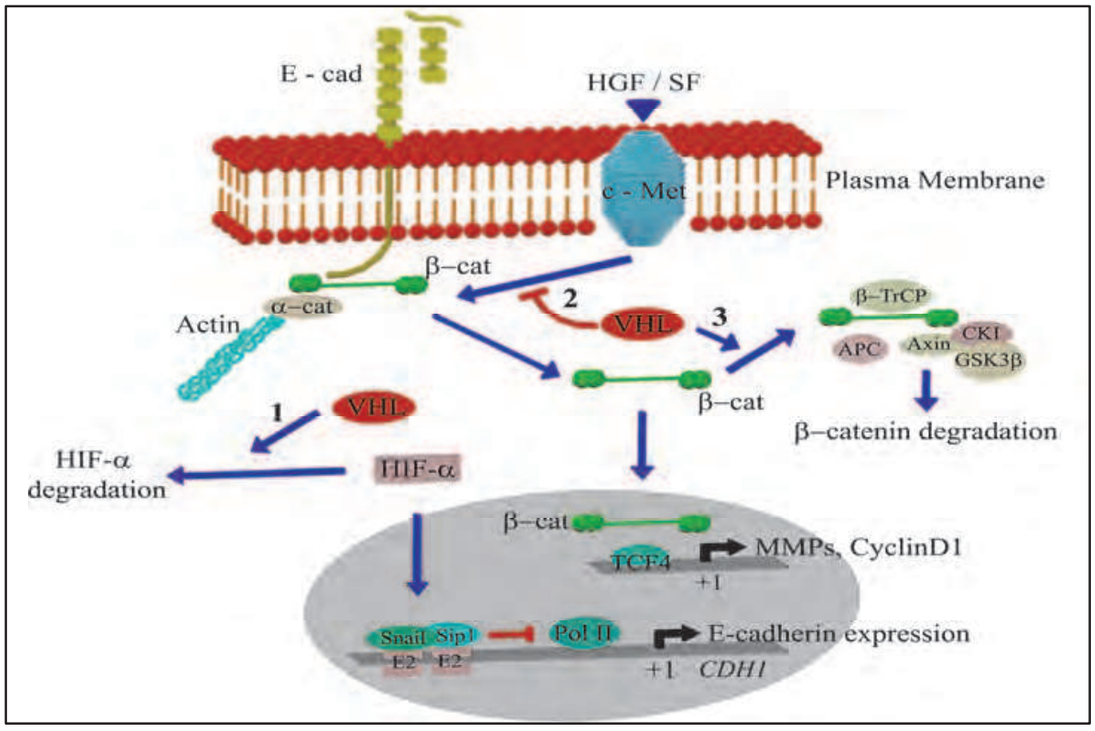


Fig. 4. Role of VHL in the regulation of E-cadherin and β-catenin. Loss of VHL leads to the stabilization of HIFα (1), which promotes the transactivation of E-cadherin-specific repressors, reducing E-caherin expression. Loss of VHL also causes constitutive phosphorylation of c-Met (2) and subsequent release, activation and stabilization of β-catenin (3).

HIF-1 and HIF-2 have diverse functions with regard to VHL renal tumourigenesis. HIF-2 has been proposed to preferentially regulate signalling pathways critical for renal cell growth, such as signalling through the TGF-α/epidermal growth factor receptor pathway and through the cell cycle regulator cyclin D1 (Bindra et al., 2002; Raval et al., 2005; Smith et al., 2005; Staller et al., 2003; Wykoff et al., 2004; Zatyka et al., 2002).

In addition to VHL-associated CC-RCC, HIF-α stabilisation can be found in renal cell cancers that are associated with mutations of the tuberous sclerosis tumour suppressor TSC-2 (Liu et al., 2003) and in rare leiomyomatosis-associated renal cancers. The latter form of renal cancer is characterised by fumarate hydratase deficiency, the inability to convert fumarate to malate, which results in HIF prolyl-hydroxylase inhibition; fumarate acts as a competitive inhibitor of HIF prolyl-hydroxylation (Isaacs et al., 2005). It is unclear whether an increase in HIF-1 and HIF-2 activity in these rare forms of renal cancer has the same biological effects as in VHL-negative CC-RCC, and further investigation is required.

5. Energy disorders in RCC

Tumours are characterised by specific metabolic alterations providing a metabolic signature in malignant transformation at different stages; end stage carcinomas are most dependent on anaerobic glucose degradation (aerobic glycolysis, fermentation) and least dependent on mitochondrial energy supplies. The metabolic endpoint of this transformation is the anaerobic degradation of glucose even in the presence of oxygen (Koppenol et al., 2011).Concomitant with this metabolic switch, high lactate concentrations occur and result in the immune protection of cancer cells, acid-mediated matrix degradation, invasiveness and metastasis (Hutterer et al., 2007).

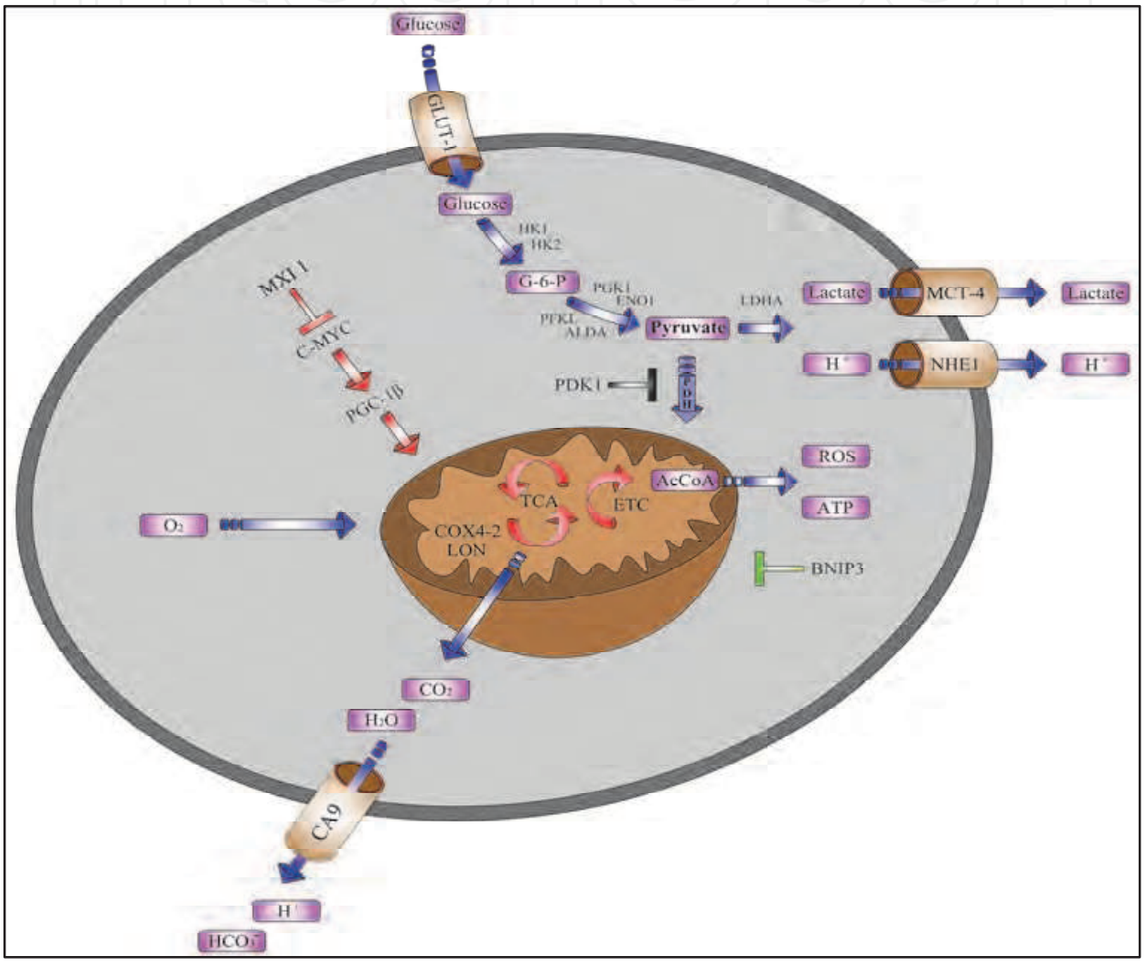


Fig. 5. Regulation of proteins required for glucose uptake and energy metabolism by HIF-1. Metabolic substrates and products are shown in purple and HIF-1-regulated gene products are shown in brown. Red arrows, mitochondrial biogenesis mediated by C-MYC (in renal carcinoma cells). Green blocked arrow, mitochondrial autophagy mediated by BNIP3 (in mouse embryo fibroblasts). AcCoA, acetyl coenzyme A; ALD, aldolase; CA9, carbonic anhydrase; COX, cytochrome c oxidase; ENO, enolase; G-6-P, glucose-6-phosphate; GLUT, glucose transporter; HK, hexokinase; LDH, lactate dehydrogenase; MCT, monocarboxylate transporter; NHE, sodium–hydrogen exchanger; PDH, pyruvate dehydrogenase; PDK, PDH kinase; PFK, phosphofructokinase; PGC, PPAR-γ co-activator; PGK, phosphoglycerate kinase.

Increased total activity of the transketolase-dependent, nonoxidative branch of the pentose phosphate pathway (PPP) in cancer cells is due to the overexpression of the transketolase-like-1 (TKTL1) protein (Hu et al., 2007).

The complex regulation of tumour metabolism switches from mitochondrial oxidative to nonoxidative energy production, which is dependent on the PPP. Both the oxidative and nonoxidative branches of the PPP have been described as activated in carcinogenesis (Ramos-Montoya et al., 2006). It is assumed that the enzymes of the oxidative branch of the PPP [glucose-6-phosphate-dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase] are triggered by an increased need for NADPH, whereas the enzymes of the nonoxidative branch (TKTL1, transaldolases) are triggered by an increased need for ribose and energy (Ramos-Montoya et al., 2006).

HIF-1 regulates the expression of hundreds of genes in human cells (Manalo et al., 2005; Elvidge et al., 2006) and is essential for embryonic development in mice (Iyer et al., 1998; Ryan et al., 1998). Many of these genes contribute to two essential functions of HIF-1. First, HIF-1 promotes the delivery of oxygen to cells through its control of erythropoiesis and angiogenesis. Second, HIF-1 promotes cell survival under hypoxic conditions by reprogramming cellular glucose and energy metabolism.

Analysis of mRNA expression in mouse embryonic stem cells that were either wild type or homozygous for a knockout allele at the *Hif1a* locus encoding the HIF-1α subunit revealed that expression of the genes encoding glucose transporters 1 and 3 and the glycolytic enzymes hexokinase 1 and 2, glucose phosphate isomerase, phosphofructokinase L, aldolase A and C, triosephosphate isomerase, phosphoglycerate kinase 1, enolase 1, pyruvate kinase M, and lactate dehydrogenase A (LDH-A) was regulated by HIF-1 (Iyer et al., 1998). In human VHL-deficient renal cell carcinoma, upregulation of GLUT1 protein expression has been demonstrated at the earliest stages of tumour formation (Mandriota et al., 2002).

The upregulation of LDH-A results in the increased conversion of pyruvate to lactate at the expense of mitochondrial utilisation of pyruvate as a substrate for pyruvate dehydrogenase (PDH), which converts pyruvate to acetyl CoA. However, recent studies have demonstrated that HIF-1 plays a direct role in actively shunting pyruvate away from the mitochondria through its regulation of the PDK1 gene (encoding PDH kinase 1) in multiple cell types, including VHL deficient renal carcinoma cells (Kim et al., 2006; Papandreou et al., 2006). Phosphorylation of the catalytic subunit of PDH by PDK1 inactivates the enzyme. In mouse embryo fibroblasts cultured from HIF-1α-null embryos, prolonged hypoxic incubation induces ROS production leading to cell death that can be rescued by the forced expression of PDK1 (Kim et al., 2006). HIF-1α-null mouse embryo fibroblasts also manifest increased cell death (relative to wild-type cells) when incubated under hypoxic conditions in the presence of the hypoxic cytotoxin tirapazamine (Papandreou et al., 2006). These results suggest that HIF-1 actively inhibits the oxidative metabolism of glucose under hypoxic conditions.

The decrease in oxygen consumption under moderate hypoxia is likely to be an adaptive mechanism to avoid the development of anoxia. During hypoxia, cells that fail to decrease their oxygen consumption are likely to become anoxic faster than cells that can suppress their rate of oxygen consumption (Denko 2008).

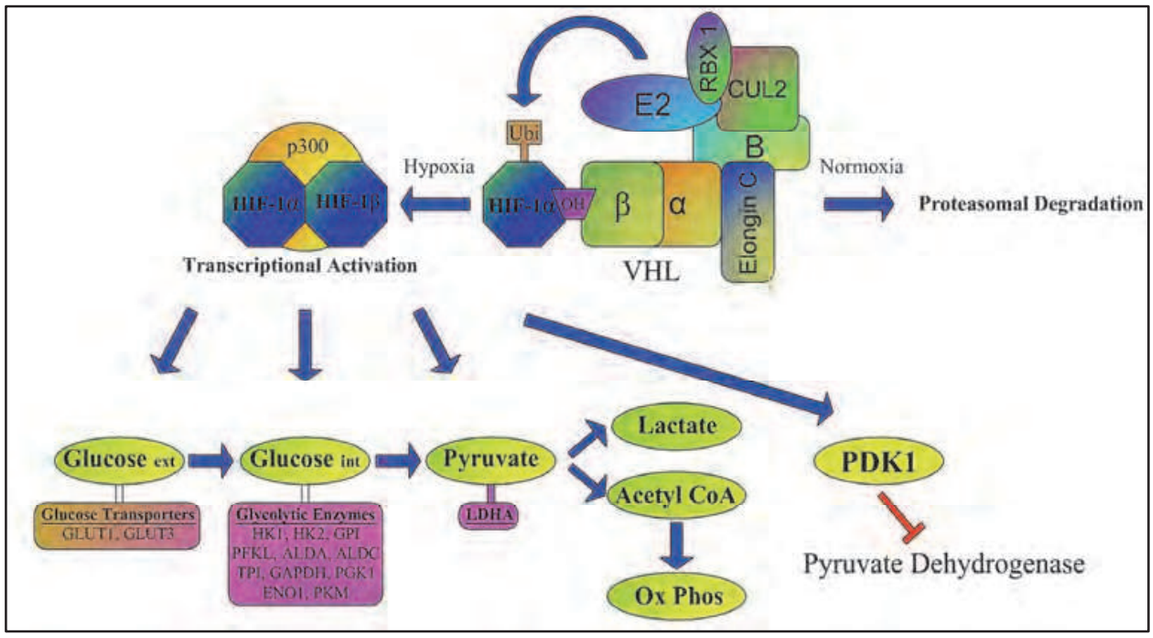


Fig. 6. Regulation of glucose metabolism by HIF-1. Under normoxic conditions, HIF-1α (or HIF-2α) is hydroxylated by PHD2, bound by VHL, ubiquitinated by an E3 ligase complex containing elongin B, elongin C, cullin 2, and Rbx1, and degraded by the proteasome. VHL loss-of-function (in clear cell renal carcinoma) or hypoxic conditions leads to the accumulation of non-hydroxylated, non-ubiquitinated HIF-1α (or HIF-2α), which dimerizes with HIF-1β, recruits the coactivator p300 (or CBP) and activates the transcription of genes encoding glucose transporter (GLUT) 1 and 3, hexokinase (HK) 1 and 2, glucosephosphate isomerase (GPI), phosphofructokinase (PFK) L, aldolase (ALD) A and C, triosephosphate isomerase (TPI), glyceraldephosphate dehydrogenase (GAPDH), phosphoglycerate kinase (PGK) 1, enolase (ENO) 1, pyruvate kinase (PK) M, lactate dehydrogenase (LDH) A, and pyruvate dehydrogenase kinase (PDK) 1.

Mitochondrial respiration in cells is controlled by cellular ATP utilisation. One model suggests that increased cytoplasmic ATP utilisation decreases cytosolic ATP levels and increases cytosolic ADP and Pi levels (Chance et al., 1955). The rise in cytosolic ADP levels leads to a rise in mitochondrial ADP via the increased activity of the adenine nucleotide carrier. The increased mitochondrial ADP concentration stimulates ATP synthase to augment the rate of ATP synthesis. The increased ATP synthesis results in a decrease in the mitochondrial membrane potential, which stimulates the respiratory chain to consume oxygen. Aside from cellular ATP utilisation, the other factors that control mitochondrial respiration are the NADH supply, the respiratory chain, and the degree of proton leak (Jones et al., 1993). The major reason for the decrease in respiration during hypoxia is the decrease in cellular ATP utilisation.

A major ATP consumer that hypoxia inhibits is Na/K-ATPase. The activity of Na/K-ATPase alone can account for 20–70% of the oxygen expenditure of mammalian cells (Milligan et al., 1985). Na/K-ATPase is a transmembrane protein found in higher eukaryotes that transports Na^+ and K^+ across the plasma membrane to maintain ionic gradients (Skou

1957). Na/K-ATPase is a heterodimer composed of α and β subunits (Kaplan 2002). The hypoxia-induced decrease of Na/K-ATPase activity is due to endocytosis of the α subunit from the plasma membrane by PKC zeta. Hypoxia stimulates the AMPK alpha-1 isoform, which directly phosphorylates PKC zeta to promote Na/K-ATPase endocytosis (Gusarova et al., 2009).

The other major ATP consumer that hypoxia inhibits is mRNA translation (Wouters et al., 2005). The mammalian target rapamycin (mTOR) and pancreatic eIF2α kinase(PERK) are the key regulators of translation during hypoxia (Arsham et al., 2003; Koumenis et al., 2002). In growth-promoting conditions, mTOR sustains translation by phosphorylating eIF4E-binding proteins (4EBPs) and ribosomal protein S6 kinases (S6Ks) (Gingras et al., 2001). Hypoxia (1.5% O_2) causes rapid and reversible hypophosphorylation of mTOR and its effectors 4E-BP1 and S6K (Arsham et al., 2003). The rapid inhibition of mTOR is HIF independent and occurs through the activation of AMPK76. Rapid activation of AMPK during hypoxia is dependent on mitochondrial ROS (Emerling et al., 2009). The other major contributor to the decrease in mRNA translation during hypoxia is the activation of PERK73, resulting in eIF2α phosphorylation and the inhibition of mRNA translation initiation. Mitochondrial ROS have been implicated in the activation of PERK.

Mitochondrial respiration is regulated by the availability of oxygen, ADP and reducing equivalents (NADH and $FADH_2$ from the TCA cycle). Oxygen is limiting for respiration under severely hypoxic conditions (<0.5% O_2). Thus, under physiological hypoxia (1–3% O_2), oxygen is not limiting for maximal respiration. The major controller of mitochondrial respiration is ADP availability from cellular ATP utilisation. Hypoxia through mitochondrial ROS also diminishes the activity of Na/K-ATPase and mRNA translation. This results in a decrease in cellular ATP utilisation and a decrease in ADP availability to mitochondria. Hypoxia also stimulates the release of mitochondrial ROS from complex III to activate HIF-1, which induces the transcription of PDK1. PDK1 negatively regulates PDH, an enzyme that converts pyruvate to acetyl-CoA. Thus, an increase in HIF-1 dependent PDK1 expression results in diminished availability of acetyl-CoA. This also contributes to diminished respiration during hypoxia by decreasing TCA cycle activity.

Although HIF-1 and HIF-2 are activated by similar mechanisms by hypoxia, they have both overlapping and distinct gene targets. HIF-1 regulates metabolic genes, whereas HIF-2 regulates EPO and the mitochondrial matrix protein superoxide dismutase 2 (SOD2). Depending on the genetic background, the loss of HIF-2 in adult mice results in profound anaemia (Gruber et al., 2007) or multiple organ pathology due to increased oxidative stress (Scortegagna M., 2003). The role of HIF-2 regulation of oxidative stress is further supported by the observation that SOD2 levels are markedly increased in VHL-null renal cell carcinoma cells (Hervouet et al., 2008). It will be interesting to determine whether activation of HIF-2 prevents oxidative stress-induced injury in other organs, such as brain, lung and heart.

6. Nutrient disorders in RCC

Diets rich in fruits and vegetables have typically been shown to decrease RCC risk, possibly through antioxidant effects, while consumption of fried foods has commonly been shown to

increase risk, possibly due to the potential carcinogenic effects of acrylamides (Chow et al., 2008). Recently, epidemiological studies suggest that vitamin D, which is found in food (vitamin D2 and D3) and produced in the body after exposure to ultraviolet (UV) rays from the sun (vitamin D3), may be inversely associated with RCC risk (Karami et al., 2008). Both vitamin D2 and D3 are hydroxylated in the liver and subsequently in the kidney to form active vitamin D (1,25(OH)2D3). Dietary intake of vitamin D accounts for a small (approximately 10%) proportion of vitamin D levels (Holick 2006). However, dietary intake of vitamin D and vitamin D rich foods may play a role in determining RCC risk, given that vitamin D has been associated with anticarcinogenic properties and is primarily metabolised within the kidneys (Holick 2006). Vitamin D and its metabolites are thought to impede carcinogenesis by stimulating cell differentiation, inhibiting cell proliferation, inducing apoptosis, and suppressing invasiveness, angiogenesis, and metastasis (Valdivielso et al., 2006). Vitamin D activation is mediated by binding to vitamin D receptors (VDRs), transcription factors that are part of the nuclear hormone receptor family. Forming a heterodimer complex with the retinoid-X-receptor (RXR) gene, VDR can regulate the transcription of other genes involved in cell regulation, growth, and immunity (Valdivielso et al., 2006; Thibault et al., 2006). Most epidemiological studies have focused on the VDR gene; however, a recent evaluation of 139 single nucleotide polymorphisms (SNPs) across eight genes in the vitamin D pathway found a significant association between RCC risk and certain VDR and RXRA genetic variants (Karami et al., 2009).

The kidney is the most important organ for vitamin D metabolism and activity as well as calcium homeostasis. Within the kidneys, calcium has been shown to influence active vitamin D levels (Holick et al., 2006). Thus, investigations of dietary vitamin D and calcium in RCC aetiology are highly relevant. A Canadian RCC case-control study showed no association for the intake of individual foods rich in vitamin D (i.e., fish and eggs) or calcium (i.e., milk, dairy, and cheese) (Hu et al., 2003). In contrast, calcium supplement intakes were shown to significantly reduce RCC risk as the number of years of intake increased. Different polymorphisms in the VDR gene have been speculated to result in variations of VDR expression and changes to circulating levels of active vitamin D (Ikuyama et al., 2002). For this reason, epidemiological studies suggest that tissue specific expression of vitamin D pathway genes function as the primary mechanism involved in linking vitamin D status with the anticarcinogenic effects of 1,25(OH)2D3. Therefore, a lower renal cancer risk may be associated with higher circulating levels of 25(OH)D, the storage form of vitamin D, by providing substrates for renal tissue-specific synthesis of 1,25(OH)2D3 (McCullough et al., 2008). RXRA, on the other hand, may play a critical role in vitamin D activity, particularly from dietary sources, since this gene has been shown to regulate cholesterol (Hegele et al., 2001), which is abundant in eggs and yogurt, the food groups that were statistically associated with renal cancer risk in this study. RXRA regulated fatty acid and cholesterol metabolism through intestinal cholesterol absorption and bile acid synthesis (Hegele et al., 2001). Cholesterol metabolism has been associated with atherosclerosis, which is associated with hypertension and cardiovascular risk, known risk factors of RCC.

Antioxidant-rich foods have several preventive effects against different diseases, such as cancer, coronary disease, inflammatory disorders, and neurologic degeneration. Honey has been used as a traditional food source since ancient times. It is made when the nectar and

sweet deposits from plants are gathered, modified, and stored in the honeycomb by honeybees. The major components of honey are fructose and glucose; honey also contains carbohydrates, proteins, amino acids, vitamins, water, minerals, and enzymes. In general, honey is also rich in antioxidants and has antibacterial properties (Brudzynski 2006). Honey not only promotes growth of new skin tissue by creating a moist environment but also prevents infection by way of its antimicrobial properties. Moreover, honey is harmless; in fact, it enables faster healing of the wounds by forming new tissues. Honey is thought to exhibit a broad spectrum of therapeutic properties, including antibacterial, antifungal, cytostatic, and anti-inflammatory activity (Jeddar et al., 1985). Honey potentiated the antitumour activity of chemotherapeutic drugs, such as 5-fluorouracil and cyclophosphamide, and contains many biologically active compounds, including caffeic acid, caffeic acid phenethyl ester, and flavonoid glycones. These compounds have been shown to have an inhibitory effect on tumour cell proliferation and transformation by the downregulation of many cellular enzymatic pathways, including protein tyrosine kinase, cyclooxygenase, and ornithine decarboxylase pathways (Chinthalapally et al., 1993).

Honey is also a dietary source for flavonoids, which have been demonstrated to have anticarcinogenic and anti-inflammatory activities. Although crude honey was reported by some authors to be a proliferative agent that enhances the proliferation of both normal and malignant cells (Rady 2005), it was also reported to be a promising antitumour agent with pronounced antimetastatic effects (Orsolic et al., 2005). The proliferative effect of honey on tumour cells was suggested to be a nutritional effect rather than a carcinogenic effect, and the antitumour effect was reported to result from many activities, such as the inhibition of DNA synthesis with no signs of cytotoxicity and the downregulation of MMP-2 and MMP-9, which have been implicated in the induction of the angiogenic switch in different model systems (Egeblad et al., 2002). Honey has cytotoxic activity against carcinomic human kidney cells, indicating that honey possesses antitumour and anticarcinogenic activities (Abdel Aziz et al., 2009).

Leptin is an adipocyte-derived hormone/cytokine that links nutritional status with neuroendocrine and immune functions. Leptin was the first adipocyte-derived hormone described, and the amount of leptin produced is directly proportional to the amount of adipose tissue. Leptin activates the anorexigenic axis in the hypothalamus. Leptin is a key intermediary between energy homeostasis and the immune system, and it may play roles in inflammation and obesity-related diseases, including atherosclerosis and cancer. The characterisation of leptin functions and signalling pathways in regulating lipid metabolism, inflammatory mediator production and lipid body biogenesis in macrophages and other cells are important for understanding the roles of leptin in the pathogenesis of inflammatory diseases.

The mTOR kinase pathway is a well-studied and evolutionarily conserved intracellular nutrient-sensing pathway (Lindsley et al., 2004). This pathway integrates nutrient- and growth factor-derived signals to set overall growth rates, and interfaces with the cell cycle machinery to coordinate cell growth and division (Richardson et al., 2004).

New findings are starting to unveil an important role for mTOR in leptin signalling, in the hypothalamic centres as well as in peripheral cells (Cota et al., 2006).

The activation of mTOR complex 1 in the hypothalamus is important for the effects of leptin on the hypothalamic axis, which modulates food intake (Cota et al., 2006). These authors showed that the activation of the mTOR pathway occurs in response to leptin stimulation and that rapamycin inhibits the anorexigenic signal of leptin. Moreover, the amino acid leucine directly activates mTOR in the hypothalamus, functioning as a redundant signal with leptin. Several nutrient sensing mediators that activate mTOR in adipose tissue, including insulin, leucine and UDP-N-acetylglucosamine, also induce leptin synthesis (Lindsley et al., 2004). The role of leptin in regulating lipid accumulation and foam cell formation in macrophages is beginning to be characterised and involves key steps mediated by mTOR-dependent signalling. Typically, increases in the size and numbers of lipid bodies are accompanied by accumulation of triacylglycerides and cholesterol esters in their hydrophobic core. In different cell systems, including adipocytes and macrophages, intracellular lipids are stored and metabolised in hydrophobic organelles called lipid bodies or lipid droplets. Although lipid bodies were long considered inert fat depots, lipid bodies are now viewed as dynamic organelles with roles in integrating lipid metabolism, inflammatory mediator production, membrane trafficking and intracellular signalling (Wang et al., 2007). Accordingly, the regulatory mechanisms of lipid body biogenesis and functions are of major interest for the study of atherosclerosis, obesity, cancer and other inflammatory diseases. In addition, the mTOR inhibitor rapamycin upregulates the expression of genes that promote fatty acid oxidation, while downregulating genes that participate in fatty acid synthesis (Peng et al., 2002). However, it should be noted that depending on the cell type and model system used, leptin might have opposite effects on intracellular lipid storage and metabolism (O´Rourke et al., 2002). In fact, leptin diminishes lipid accumulation in the liver, kidney and adipose tissue (Motomura et al., 2006). These data point to different roles and possibly different signalling pathways for leptin, depending on the tissue.

Numerous studies have established a link between diet and cancer risk or progression; as a result, the World Cancer Research Fund has recently acknowledged that after smoking, diet may be the second most important contributor to the global burden of cancer (http://www.wcrf-uk.org/preventing cancer/index.php.).

7. Iron disorders in RCC

Iron metabolism is crucial in all aspects of energy production in the body (Anderson et al., 2009) and is particularly important for cells that are characterised by high-energy demands, such as tumourous cells. Iron has the ability to shuttle between two oxidative states (ferric and ferrous iron), which makes it an efficient cofactor for several enzymes and the catalyst of numerous biochemical reactions (Anderson et al., 2009). The ferrous form Fe(II) can donate electrons, while the Fe(III) form can accept electrons. Iron plays a crucial role in oxygen transport (as a component of hemoglobin (Hb)), oxygen storage (as a component of myoglobin), and oxidative metabolism (as a component of oxidative enzymes and respiratory chain processes).

Ion is also involved in the synthesis and degradation of lipids, carbohydrates, DNA, and RNA as well as in the metabolism of collagen, tyrosine, and catecholamines.

Therefore, iron deficiency can impair oxidative metabolism, cellular energetics, and cellular immune mechanisms.

Experimental studies in animals have shown that severe iron deficiency can cause diastolic dysfunction and heart failure with pulmonary congestion, left ventricular hypertrophy and dilation, cardiac fibrosis, a reduction in erythropoietin levels and a worsening of the molecular signalling pathways (as measured by cardiac STAT3 phosphorylation), an increase in the inflammatory cytokine TNFα, and proteinuria (Dong et al., 2005). Iron may have anti-inflammatory effects. Compared to haemodialysis patients taking EPO alone, those taking EPO and IV iron had lower proinflammatory TNFα levels and higher anti-inflammatory cytokine IL-4 levels as well as lower levels of total peroxide (a marker of free radical concentration) (Weiss et al., 2003).

The biology of iron and oxygen is closely related, and known regulatory pathways involving HIF and iron-regulatory proteins (IRPs) are responsive to both these stimuli.

In humans, iron is absorbed only in the small intestine, stored in the liver and the reticuloendothelial (RE) system, and is mainly used in bone marrow. Intestinal absorption of iron depends on four types of proteins of iron metabolism: duodenal cytochrome b (Dcytb), divalent metal transporter 1 (DMT1), ferroportin 1 (FP1) and hephaestin (Hp).

Many proteins of iron metabolism show a high degree of expression in tumour cells, indicating that iron plays an important role in tumourigenesis and development. However, regulatory factors of iron metabolism and their mechanisms remain to be studied. New treatment strategies may be developed by combining imaging agents or targeted drugs with proteins related to iron metabolism. Recently, magnetic nanoparticles carrying chemotherapeutic drugs provide a new thinking for solid tumour targeted therapy. For example, the combination of the magnetic nanoparticle Fe_3O_4 with cisplatin (DDP) is used to reverse DDP resistance in the human ovarian cancer cell line SKOV3/DDP through increasing intracellular drug concentrations and promoting cell apoptosis by reducing mRNA expression of the antiapoptosis genes *bcl2* and *survivin* (Jiang et al., 2009).

8. Oxygen sensing and RCC

Tumours are characterised by specific metabolic alterations providing a metabolic signature in malignant transformation at different stages; end stage carcinomas are most dependent on anaerobic glucose degradation (aerobic glycolysis, fermentation) and least dependent on mitochondrial energy supplies (Ramanathan et al., 2005). The metabolic endpoint of this transformation, the anaerobic degradation of glucose even in the presence of oxygen, was first described by Nobel laureate Otto Warburg (Warburg et al., 1924). Concomitant with this metabolic switch, high lactate concentrations occur and result in immune protection of cancer cells, acid-mediated matrix degradation, invasiveness and metastasis. Furthermore, transformation to a more malignant phenotype is associated with resistance to chemo- and radiation-therapy (Cao et al., 2007).

Increased total activity of the transketolase-dependent, nonoxidative branch of the pentose phosphate pathway (PPP) in cancer cells is due to the overexpression of the transketolase-like-1 (TKTL1) protein (Hu et al., 2007).

The complex regulation of tumour metabolism switches from mitochondrial oxidative to nonoxidative energy production, which is dependent on the PPP. Both the oxidative and nonoxidative branches of the PPP have been described as activated in carcinogenesis. It is assumed that the enzymes of the oxidative branch of the PPP [glucose-6-phosphate-dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase] are triggered by an increased need for NADPH, whereas the enzymes of the nonoxidative branch (TKTL1, transaldolases) are triggered by an increased need for ribose and energy (Ramos-Montoya et al., 2006).

During malignant transformation of cancer cells, a metabolic switch from mitochondrially based, oxygen-dependent ATP production (oxidative phosphorylation) to anaerobic glucose degradation (aerobic glycolysis, fermentation) leading to oxygen- and mitochondria-independent ATP generation takes place, even in the presence of oxygen (Ramanathan et al., 2005).

Activation of HIF-1, a key transcription factor that upregulates genes involved in glycolytic energy metabolism, is a common feature of RCC and has been linked to malignant transformation, metastasis and treatment resistance. In the absence of a functional von Hippel-Lindau tumour suppressor protein (70% of sporadic RCCs), irrespective of oxygen concentration, HIF-1α is not degraded and translocates to the nucleus where it dimerises with HIF-1β to form transcriptionally active HIF. HIF-1α is increased by hypoxia, insulin, insulin-like growth factor, epidermal growth factor and angiotensin II. The glycolysis-activated accumulation of HIF-1 protein once again stresses the crucial role of aerobic glycolysis in carcinogenesis and has been demonstrated to be a potent target in anticancer therapy (Oh et al., 2008).

Increased lactic-acid production and excretion by fermenting tumour cells results in suppression of cytokine production, T-cell inactivation, acid-mediated matrix degradation and apoptosis of surrounding healthy cells, leading to invasion and metastasis. Thus, high lactate production results in an exceptional growth advantage for tumour cells. The correlation between mitochondrial dysfunction and increased aerobic glycolysis in carcinogenesis has been investigated, but the competitive advantage for tumour cells is still under discussion (Ramanathan et al., 2005; Brandon et al., 2006). Mitochondrial energy production is correlated with release of reactive oxygen species (ROS) that damage proteins and macromolecules such as DNA. During proliferation, DNA is exposed to ROS that leads to severe DNA damage and mutations. Fermentative cancer cells do not produce mitochondrial ROS, thus preventing ROS-induced DNA alterations.

Anaerobic glucose metabolism is believed to have a poorer energy output in relation to the energy stored in the glucose molecule. Therefore, the elevated demand for glucose is compensated by the upregulation of glucose transporters and the onset of aerobic glycolysis in a PI-3K-dependent manner, resulting in high lactate concentrations (Walenta et al., 2004). The switch to anaerobic energy production by the TKTL1-dependent, nonoxidative branch of the PPP supports the enormous demand for (ROS-free) energy and anabolic substrates, such as ribose, NADPH and acetyl-CoA. The modified, TKTL1-dependent PPP seems a general biochemical program suitable for safe and enhanced energy release, and the anabolic substrate production necessary for rapid cell proliferation.

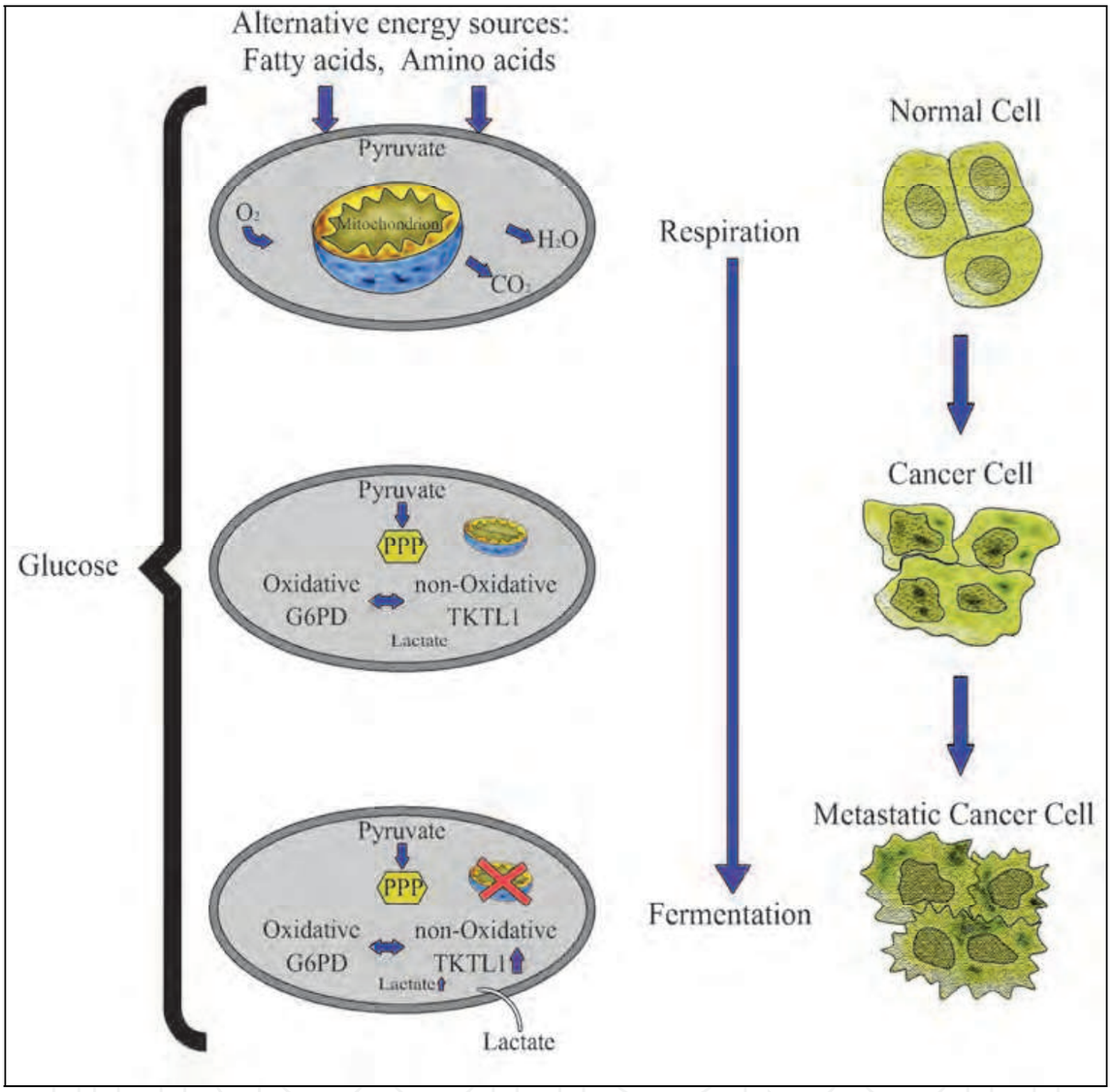


Fig. 7. Malignant transformation of cancer cells: renal tumors are present with an altered glucose metabolism. Enhanced glycolysis leads to increased activity of the enzymes in the oxidative (G6PD) and nonoxidative branches (TKTL1) of the PPP. Progressing tumors are characterized by specific upregulation of the nonoxidative branch of the PPP, ensuring ribose and energy, and supporting acidification of the tumor microenvironment. Acidification is a major step in invasive tumor growth, metastasis and immune escape. Additionally, upregulation of the anaerobic energy supply without ROS production, and G6PD activity for increased reducing equivalents, protects cancer cells from oxidative stress.

9. Conclusions

The oxygen-dependent regulation of HIF activity involves very complex pathways that involve prolyl hydroxylases and the VHL tumour suppressor protein, both of which are highly modulated by environmental and physiologic cues. The exact roles of the different HIF-α isoforms in these processes are still under intense investigation.

In the past decade, studying the regulation of HIFs has led to an appreciation that mitochondrial metabolism and ROS are essential regulators of HIFs. Conversely, HIFs have also been shown to regulate mitochondrial metabolism and ROS levels. Because mitochondria are the major consumers of oxygen, it is not surprising that HIFs and mitochondria are inter-connected.

The evidence accumulated from ecologic and case control studies show that diet has an important role in the development of RCC. Energy intake and several dietary factors should be considered as potentially involved in the development of renal cell cancer at different stages of tumourigenesis.

In this chapter, we have summarised the most recent findings in HIF signalling and have attempted to provide a perspective on how recent advances in HIF biology may affect our understanding of renal cancer.

10. References

Abdel Aziz A, Rady H, Amer MA, and Kiwan HS. (2009) Effect of Some Honey Bee Extracts on the Proliferation, Proteolytic and Gelatinolytic Activities of the Hepatocellular Carcinoma Hepg2 Cell Line. *Aus J Basic App Sci* 3, 2754–69

Anderson GJ and Vulpe CD (2009) Mammalian iron transport. *Cellular and Molecular Life Sciences* 66, 3241–3261

Arsham AM, Howell JJ, and Simon MC. (2003) A novel hypoxia-inducible factor-independent hypoxic response regulating mammalian target of rapamycin and its targets. *J Biol Chem* 278, 29655–29660

Bindra RS, Vasselli JR, Stearman R, Linehan WM, and Klausner RD. (2002) VHL-mediated hypoxia regulation of cyclin D1 in renal carcinoma cells. *Cancer Res* 62, 3014–3019

Brandon M, Baldi P, and Wallace DC. (2006) Mitochondrial mutations in cancer. *Oncogene* 25, 4647–62

Brudzynski K. (2006) Effect of hydrogen peroxide on antibacterial activities of Canadian honeys. *Can J Microbiol*. 52, 1228–37

Cao X, Fang L, Gibbs S, Huang Y, Dai Z, Wen P, Zheng X, Sadee W, and Sun D. (2007) Glucose uptake inhibitor sensitizes cancer cells to daunorubicin and overcomes drug resistance in hypoxia. *Cancer Chemother Pharmacol* 59:495–505

Chance B and Williams GR. (1955) Respiratory enzymes in oxidative phosphorylation. I. Kinetics of oxygen utilization. *J Biol Chem* 217, 383–393

Chinthalapally V, Dhimant D, Barbara S, Nalini K, Shantu A, and Bandaru R. (1993) Inhibitory effect of caffeic acid esters on azoxymethane-induced biochemical changes and aberrant crypt foci formation in rat colon. *Cancer Res* 53, 4182–8

Chow WH and Devesa SS. (2008) Contemporary epidemiology of renal cell cancer *Cancer Journal* 14, 288–301

Colgan SP, and Taylor CT. (2010) Hypoxia: an alarm signal during intestinal inflammation. *Nat Rev Gastroenterol Hepatol* 7, 281-7

Cota D, Proulx K, Smith KA, Kozma SC, Thomas G, Woods SC, and Seeley RJ.(2006) Hypothalamic mTOR signaling regulates food intake. *Science* 312, 927-30

Cramer T, Yamanishi Y, Clausen BE, Forster I, Pawlinski R, Mackman N, Haase VH, Jaenisch R, Corr M, Nizet V, Firestein GS, Gerber HP, Ferrara N, and Johnson RS.(2003) HIF-1α is essential for myeloid cell-mediated inflammation. *Cell* 112, 645–657

Denko NC. (2008) Hypoxia, HIF1 and glucose metabolism in the solid tumour. *Nat Rev Cancer* 8, 705–713

Dong F, Zhang X, Culver B, Chew HG, Kelley RO, and Ren J (2005) Dietary iron deficiency induces ventricular dilation, mitochondrial ultrastructural aberrations and cytochrome c release: involvement of nitric oxide synthase and protein tyrosine nitration. *Clinical Science* 109, 277–286

Eckle T, Grenz A, Laucher S, and Eltzschig HK. A2B adenosine receptor signaling attenuates acute lung injury by enhancing alveolar fluid clearance in mice. *J Clin Invest* 118, 3301-15

Eckle T, Köler D, Lehmann R, El Kasmi KC, and Eltzschig HK. (2008) Hypoxia-inducible factor-1 is central to cardioprotection: a new paradigm for ischemic preconditioning. *Circulation* 118, 166-75

Egeblad M, and Werb Z. (2002) New functions for the matrix metalloproteinases in cancer progression. *Nat Rev Cancer* 2, 161–74

Eltzschig HK, Ibla JC, Furuta GT, Leonard MO, Jacobson KA, Enjyoji K, Robson SC, and Colgan SP. (2003) Coordinated adenine nucleotide phosphohydrolysis and nucleoside signaling in posthypoxic endothelium: role of ectonucleotidases and adenosine A2B receptors. *J Exp Med* 198, 783-96

Eltzschig HK. (2009) Adenosine: an old drug newly discovered. *Anesthesiology* 111, 904-15

Elvidge GP, Glenny L, Appelhoff RJ, Ratcliffe PJ, Ragoussis J, and Gleadle JM (2006) Concordant regulation of gene expression by hypoxia and 2-oxoglutarate-dependent dioxygenase inhibition: the role of HIF-1alpha, HIF-2alpha, and other pathways. *J Biol Chem* 281, 15215–15226

Emerling BM, Weinberg F, Snyder C, Burgess Z, Mutlu GM, Viollet B, Budinger GR, and Chandel NS. (2009) Hypoxic activation of AMPK is dependent on mitochondrial ROS but independent of an increase in AMP/ATP ratio. *Free Radic Biol Med* 46, 1386–1391

Fukuda R, Hirota K, Fan F, Jung YD, Ellis LM, and Semenza GL. (2002) Insulin-like growth factor 1 induces hypoxia-inducible factor 1-mediated vascular endothelial growth factor expression, which is dependent on MAP kinase and phosphatidylinositol 3-kinase signaling in colon cancer cells. *J Biol Chem* 277, 38205–38211

Furuta GT, Turner JR, Taylor CT, Hershberg RM, Comerford K, Narravula S, Podolsky DK, and Colgan SP. (2001) Hypoxia-inducible factor 1-dependent induction of intestinal trefoil factor protects barrier function during hypoxia. *J Exp Med* 193, 1027-34

Gingras AC, Raught B, Gygi SP, Niedzwiecka A, Miron M, Burley SK, Polakiewicz RD, Wyslouch-Cieszynska A, Aebersold R, and Sonenberg N. (2001) Hierarchical phosphorylation of the translation inhibitor 4EBP1.*Genes Dev* 15, 2852–2864

Gruber M, Hu CJ, Johnson RS, Brown EJ, Keith B, and Simon MC. (2007) Acute postnatal ablation of Hif-2alpha results in anemia. *Proc Natl Acad Sci U S A*. 104, 2301–2306

Gusarova GA, Dada LA, Kelly AM, Brodie C, Witters LA, Chandel NS, and Sznajder JI. (2009) Alpha1-AMP-activated protein kinase regulates hypoxia-induced Na,K-ATPase endocytosis via direct phosphorylation of protein kinase C zeta. *Mol Cell Biol* 29, 3455–3464

Gustafsson MV, Zheng X, Pereira T, Gradin K, Jin S, Lundkvist J, Ruas JL, Poellinger L, Lendahl U, and Bondesson M. (2005) Hypoxia requires notch signaling to maintain the undifferentiated cell state. *Dev Cell* 9, 617–628

Haase VH. (2005) The VHL tumor suppressor in development and disease: functional studies in mice by conditional gene targeting. *Semin Cell Dev Biol* 16, 564–574

Hegele RA and Cao H. (2001) Single nucleotide polymorphisms of RXRA encoding retinoid X receptor alpha. *Journal of Human Genetics* 46, 423–425

Hervouet E, Cízková A, Demont J, Vojtísková A, Pecina P, Franssen-van Hal NL, Keijer J, Simonnet H, Ivánek R, Kmoch S, Godinot C, and Houstek J. (2008) HIF and reactive oxygen species regulate oxidative phosphorylation in cancer. Carcinogenesis 29, 1528–1537

Holick MF (2006) Vitamin D: its role in cancer prevention and treatment *Progress in Biophysics and Molecular Biology* 92, 49–59

Hu CJ, Wang LY, Chodosh LA, Keith B, and Simon MC. (2003) Differential roles of hypoxia-inducible factor 1α (HIF-1α) and HIF-2α in hypoxic gene regulation. *Mol Cell Biol* 23, 9361–9374

Hu J, Mao Y, and White K. (2003) Diet and vitamin or mineral supplements and risk of renal cell carcinoma in Canada. *Cancer Causes Control* 14, 705–714

Hu LH, Yang JH, Zhang DT, Zhang S, Wang L, Cai PC, Zheng JF, and Huang JS. (2007) The TKTL1 gene influences total transketolase activity and cell proliferation in human colon cancer LoVo cells. *Anticancer Drugs* 18, 427–33

Hu LH, Yang JH, Zhang DT, Zhang S, Wang L, Cai PC, Zheng JF, and Huang JS. (2007) The TKTL1 gene influences total transketolase activity and cell proliferation in human colon cancer LoVo cells. *Anticancer Drugs* 18, 427–33

Hutterer GC, Patard JJ, Perrotte P, Ionescu C, de La Taille A, Salomon L, Verhoest G, Tostain J, Cindolo L, Ficarra V, Artibani W, Schips L, Zigeuner R, Mulders PF, Valeri A, Chautard D, Descotes JL, Rambeaud JJ, Mejean A, and Karakiewicz PI. (2007) Patients with renal cell carcinoma nodal metastases can be accurately identified: external validation of a new nomogram. *Int J Cancer* 121, 2556–61

Ikuyama T, Hamasaki T, Inatomi H, Katoh T, Muratani T, and Matsumoto T. (2002) Association of vitamin D receptor gene polymorphism with renal cell carcinoma in Japanese *Endocrine Journal* 49, 433–438

Isaacs JS, Jung YJ, Mole DR, Lee S, Torres-Cabala C, Chung YL, Merino M, Trepel J, Zbar B, Toro J, Ratcliffe PJ, Linehan WM, and Neckers L. (2005) HIF overexpression

correlates with biallelic loss of fumarate hydratase in renal cancer: novel role of fumarate in regulation of HIF stability. *Cancer Cell* 8, 143–153

Iyer NV, Kotch LE, Agani F, Leung SW, Laughner E, Wenger RH, Gassmann M, Gearhart JD, Lawler AM, Yu AY, and Semenza GL (1998) Cellular and developmental control of O2 homeostasis by hypoxia-inducible factor 1 alpha. *Genes Dev* 12, 149–162

Jeddar A, Khassany A, Ramsaroop VG, Bhamjei IE, Moosa A. (1985) The antibacterial action of honey: an in vitro study. *S Afr Med J* 67, 257–9

Jiang BH, Jiang G, Zheng JZ, Lu Z, Hunter T, and Vogt PK. (2001) Phosphatidylinositol 3-kinase signaling controls levels of hypoxia-inducible factor 1. *Cell Growth Differ* 12, 363–369

Jiang Z, Chen BA, Wu Q, Xia GH, Zhang Y, Gao F, Hong TY, Xu CR, Cheng J, Li GH, Chen WJ, Liu LJ, Li XM, and Wang XM. (2009) Reversal effect of Fe3O4-magnetic nanoparticles on multi-drug resistance of ovarian carcinoma cells and its correlation with apoptosis-associated genes *J Chin J Cancer* 28, 1158-1162

Jones DP, Shan X and Park Y. (1993) Coordinated multisite regulation of cellular energy metabolism. *Annu Rev Nutr* 12, 327–343

Kaelin WG. Proline hydroxylation and gene expression. (2005) *Annu Rev Biochem* 74, 115–128

Kanehisa M, Goto S, Furumichi M, Tanabe M, and Hirakawa M. (2010) KEGG for representation and analysis of molecular networks involving diseases and drugs. *Nucleic Acids Res.* 38, D355-D360

Kaplan JH. (2002) Biochemistry of Na,K-ATPase. *Annu Rev Biochem* 71, 511–535

Karami S, Brennan P, Hung RJ, Boffetta P, Toro J, Wilson RT, Zaridze D, Navratilova M, Chatterjee N, Mates D, Janout V, Kollarova H, Bencko V, Szeszenia-Dabrowska N, Holcatova I, Moukeria A, Welch R, Chanock S, Rothman N, Chow WH, and Moore LE. (2008)Vitamin D receptor polymorphisms and renal cancer risk in Central and Eastern Europe *Journal of Toxicology and Environmental Health A* 6, 367–372

Karami S, Brennan P, Rosenberg PS, Navratilova M, Mates D, Zaridze D, Janout V, Kollarova H, Bencko V, Matveev V, Szeszenia-Dabrowska N, Holcatova I, Yeager M, Chanock S, Menashe I, Rothman N, Chow WH, Boffetta P, and Moore LE. (2009) Analysis of SNPs and haplotypes in vitamin D pathway genes and renal cancer risk. PLoS ONE 4, e7013

Karhausen J, Furuta GT, Tomaszewski JE, Johnson RS, Colgan SP, Haase VH. (2004) Epithelial hypoxia-inducible factor-1 is protective in murine experimental colitis. *J Clin Invest* 114, 1098-106

Kempf VA, Lebiedziejewski M, Alitalo K, Wälzlein JH, Ehehalt U, Fiebig J, Huber S, Schütt B, Sander CA, Müller S, Grassl G, Yazdi AS, Brehm B, and Autenrieth IB. (2005) Activation of hypoxia-inducible factor-1 in bacillary angiomatosis: evidence for a role of hypoxia-inducible factor- 1 in bacterial infections. *Circulation* 111, 1054-62

Kim J-W, Tchernyshyov I, Semenza GL, and Dang CV (2006) HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. *Cell Metab* 3, 177–185

Kong T, Eltzschig HK, Karhausen J, Colgan SP, and Shelley CS. (2004) Leukocyte adhesion during hypoxia is mediated by HIF-1-dependent induction of β2 integrin gene expression. *Proc Natl Acad Sci USA* 101, 10440–10445

Koppenol WH, Bounds PL, and Dang CV. (2011) Otto Warburg's contributions to current concepts of cancer metabolism. *Nat Rev Cancer* 11, 325-37

Koshiji M, Kageyama Y, Pete EA, Horikawa I, Barrett JC, and Huang LE. (2004) HIF-1α induces cell cycle arrest by functionally counteracting Myc. *EMBO J* 23, 1949–1956

Koumenis C, Naczki C, Koritzinsky M, Rastani S, Diehl A, Sonenberg N, Koromilas A, and Wouters BG. (2002) Regulation of protein synthesis by hypoxia via activation of the endoplasmic reticulum kinase PERK and phosphorylation of the translation initiation factor eIF2alpha. *Mol Cell Biol* 22, 7405–7416

Lindsley JE, and Rutter J. (2004) Nutrient sensing and metabolic decisions. *Comp Biochem Physiol B Biochem Mol Biol* 139, 543-59

Liu MY, Poellinger L, and Walker CL. (2003) Up-regulation of hypoxiainducible factor 2α in renal cell carcinoma associated with loss of Tsc-2 tumor suppressor gene. *Cancer Res* 63, 2675–2680

Manalo DJ, Rowan A, Lavoie T, Natarajan L, Kelly BD, Ye SQ, Garcia JG, and Semenza GL (2005) Transcriptional regulation of vascular endothelial cell responses to hypoxia by HIF-1. *Blood* 105, 659–669

Mandriota SJ, Turner KJ, Davies DR, Murray PG, Morgan NV, Sowter HM, Wykoff CC, Maher ER, Harris AL, Ratcliffe PJ, and Maxwell PH (2002) HIF activation identifies early lesions in VHL kidneys: evidence for site-specific tumor suppressor function in the nephron. *Cancer Cell* 1, 459–468

McCullough ML, Bandera EV, Moore DF, and Kushi LH. (2008) Vitamin D and calcium intake in relation to risk of endometrial cancer: a systematic review of the literature. *Prev Med* 46, 298-302

Morote-Garcia JC, Rosenberger P, Kuhlicke J, and Eltzschig HK. (2008) HIF-1-dependent repression of adenosine kinase attenuates hypoxia-induced vascular leak. *Blood* 111, 5571-80

Motomura W, Inoue M, Ohtake T, Takahashi N, Nagamine M, Tanno S, Kohgo Y, and Okumura T. (2006) Upregulation of ADRP in fatty liver in human and liver steatosis in mice fed with high fat diet. *Biochem Biophys Res Commun* 340, 1111-8

Multiple organ pathology, metabolic abnormalities and impaired homeostasis of reactive oxygen species in Epas1-/- mice. (2003) Multiple organ pathology, metabolic abnormalities and impaired homeostasis of reactive oxygen species in Epas1−/− mice. *Nat Genet* 35, 331–340

O'Rourke L, Gronning LM, Yeaman SJ, and Shepherd PR. (2002) Glucose-dependent regulation of cholesterol ester metabolism in macrophages by insulin and leptin. *J Biol Chem* 277, 42557-62

Oh SH, Woo JK, Jin Q, Kang HJ, Jeong JW, Kim KW, Hong WK, and Lee HY. (2008) Identification of novel antiangiogenic anticancer activities of deguelin targeting hypoxia-inducible factor-1a. *Int J Cancer* 122, 5–14

Orsolic N, Terzic S, Sver L, and Basic I. (2005) Honey-bee products in prevention and/or therapy of murine transplantable tumours. *J Sci Food Agri* 85, 363–70

Papandreou I, Cairns RA, Fontana L, Lim AL, and Denko NC (2006) HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. *Cell Metab* 3, 187-197

Peng T, Golub TR, and Sabatini DM. (2002) The immunosuppressant rapamycin mimics a starvation-like signal distinct from amino acid and glucose deprivation. *Mol Cell Biol* 22, 5575-84

Rady H. (2005) Faculty of Science. Cairo University; Phytochemical and biological study of an antitumor agent of plant origin mixed withhoney on malignant human cells in vitro.

Ramanathan A, Wang C, and Schreiber SL. (2005) Perturbational profiling of a cell-line model of tumorigenesis by using metabolic measurements. *Proc Natl Acad Sci USA* 102, 5992–7

Ramos-Montoya A, Lee WN, Bassilian S, Lim S, Trebukhina RV, Kazhyna MV, Ciudad CJ, Noe V, Centelles JJ, and Cascante M. (2006) Pentose phosphate cycle oxidative and nonoxidative balance: a new vulnerable target for overcoming drug resistance in cancer. *Int J Cancer* 119, 2733–41

Ramos-Montoya A, Lee WN, Bassilian S, Lim S, Trebukhina RV, Kazhyna MV, Ciudad CJ, Noe V, Centelles JJ, and Cascante M. (2006) Pentose phosphate cycle oxidative and nonoxidative balance: a new vulnerable target for overcoming drug resistance in cancer. *Int J Cancer* 119, 2733–41

Rankin EB, Higgins DF, Walisser JA, Johnson RS, Bradfield CA, and Haase VH. (2005) Inactivation of the arylhydrocarbon receptor nuclear translocator (Arnt) suppresses von Hippel-Lindau disease-associated vascular tumors in mice. *Mol Cell Biol* 25, 3163–3172

- Raval RR, Lau KW, Tran MG, Sowter HM, Mandriota SJ, Li JL, Pugh CW, Maxwell PH, Harris AL, and Ratcliffe PJ. (2005) Contrasting properties of hypoxia-inducible factor 1 (HIF-1) and HIF-2 in von Hippel-Lindau-associated renal cell carcinoma. *Mol Cell Biol* 25, 5675–5686

Reutershan J, Vollmer I, Stark S, Wagner R, Ngamsri KC, and Eltzschig HK. (2009) Adenosine and inflammation: CD39 and CD73 are critical mediators in LPS-induced PMN trafficking into the lungs. *FASEB J* 23, 473-82

Richard DE, Berra E, and Pouyssegur J. (2000) Nonhypoxic pathway mediates the induction of hypoxia-inducible factor 1α in vascular smooth muscle cells. *J Biol Chem* 275, 26765–26771

Richardson CJ, Schalm SS, and Blenis J. (2004) PI3-kinase and TOR: PIKTORing cell growth. *Semin Cell Dev Biol* 15, 147-59

Robinson A, Keely S, Karhausen J, Gerich ME, Furuta GT, and Colgan SP. (2008) Mucosal protection by hypoxia-inducible factor prolyl hydroxylase inhibition. *Gastroenterology* 134, 145-55

Rosenberger C, Mandriota S, Jurgensen JS, Wiesener MS, Horstrup JH, Frei U, Ratcliffe PJ, Maxwell PH, Bachmann S, and Eckardt KU. (2002) Expression of hypoxia-inducible factor-1α and -2α in hypoxic and ischemic rat kidneys. *J Am Soc Nephrol* 13, 1721–1732

Rosenberger P, Schwab JM, Mirakaj V, Masekowsky E, Mager A, Morote-Garcia JC, Unertl K, and Eltzschig HK. (2009) Hypoxia-inducible factor-dependent induction of netrin-1 dampens inflammation caused by hypoxia. *Nat Immunol* 10, 195-202

Ryan HE, Lo J, and Johnson RS (1998) HIF-1 alpha is required for solid tumor formation and embryonic vascularization. *EMBO J* 17, 3005–3015

Sandau KB, Zhou J, Kietzmann T, and Brune B. (2001) Regulation of the hypoxia-inducible factor 1α by the inflammatory mediators nitric oxide and tumor necrosis factor-α in contrast to desferroxamine and phenylarsine oxide. *J Biol Chem* 276, 39805–39811

Schingnitz U, Hartmann K, Macmanus CF, Eckle T, Zug S, Colgan SP, Eltzschig HK. (2010) Signaling through the A2B adenosine receptor dampens endotoxininduced acute lung injury. *J Immunol* 184, 5271-9

Schofield CJ and Ratcliffe PJ. (2004) Oxygen sensing by HIF hydroxylases. *Nat Rev Mol Cell Biol* 5, 343–354

Semenza GL. (2003) Targeting HIF-1 for cancer therapy. *Nat Rev Cancer* 3, 721-32

Skou JC. (1957) The influence of some cations on an adenosine triphosphatase from peripheral nerves. *Biochim Biophys Acta* 23, 394–401

Smith K, Gunaratnam L, Morley M, Franovic A, Mekhail K, and Lee S. (2005) Silencing of epidermal growth factor receptor suppresses hypoxiainducible factor-2-driven VHL-/- renal cancer. *Cancer Res* 65: 5221–5230

Soilleux EJ, Turley H, Tian YM, Pugh CW, Gatter KC, and Harris AL. (2005) Use of novel monoclonal antibodies to determine the expression and distribution of the hypoxia regulatory factors PHD-1, PHD-2, PHD-3 and FIH in normal and neoplastic human tissues. *Histopathology* 47, 602–610

Staller P, Sulitkova J, Lisztwan J, Moch H, Oakeley EJ, and Krek W. (2003) Chemokine receptor CXCR4 downregulated by von Hippel-Lindau tumour suppressor pVHL. *Nature* 425, 307–311

The Word Cancer Resarch Fund, June 2009, http://www.wcrf-uk.org/preventing cancer/index.php.

Thibault F, Cancel-Tassin G, and Cussenot O. (2006) Low penetrance genetic susceptibility to kidney cancer *BJU International* 98, 735–738

Treins C, Giorgetti-Peraldi S, Murdaca J, Semenza GL, and Van Obberghen E. (2002) Insulin stimulates hypoxia-inducible factor 1 through a phosphatidylinositol 3-kinase/target of rapamycin-dependent signalling pathway. *J Biol Chem* 277: 27975–27981

Valdivielso JM and Fernandez E. (2006) Vitamin D receptor polymorphisms and diseases *Clinica Chimica Acta* 371, 1–12

Walenta S, and Mueller-Klieser WF. (2004) Lactate: mirror and motor of tumor malignancy. *Semin Radiat Oncol* 14, 267–74

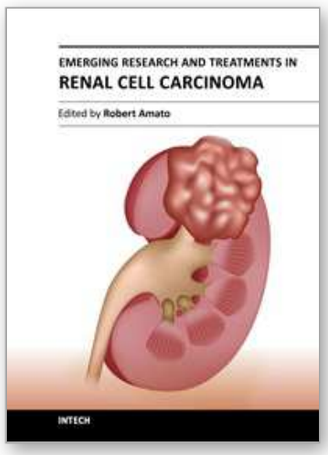
Warburg O, Posener K, and Negelein E. (1924) Über den Stoffwechsel der Carcinomcelle. *Biochem* 152, 309–44

Weiss G, Meusburger E, Radacher G, Garimorth K, Neyer U, and Mayer G. (2003) Effect of iron treatment on circulating cytokine levels in ESRD patients receiving recombinant human erythropoietin. *Kidney International* 64, 572–578

Wouters BG, van den Beucken T, Magagnin MG, Koritzinsky M, Fels D, and Koumenis C. (2005) Control of the hypoxic response through regulation of mRNA translation. *Semin Cell Dev Biol* 16, 487–501

Wykoff CC, Sotiriou C, Cockman ME, Ratcliffe PJ, Maxwell P, Liu E, and Harris AL. (2004) Gene array of VHL mutation and hypoxia shows novel hypoxia-induced genes and that cyclin D1 is a VHL target gene. *Br J Cancer* 90, 1235–1243

Zatyka M, da Silva NF, Clifford SC, Morris MR, Wiesener MS, Eckardt KU, Houlston RS, Richards FM, Latif F, and Maher ER. (2002) Identification of cyclin D1 and other novel targets for the von Hippel-Lindau tumor suppressor gene by expression array analysis and investigation of cyclin D1 genotype as a modifier in von Hippel-Lindau disease. *Cancer Res* 62, 3803–3811



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The field of renal cell cancer has undergone a significant resurgence. This book summarizes up-to-date research and innovative ideas for the future in this rapidly changing field, which encompasses medicine, surgery, radiation oncology, basic science, pathology, radiology, and supportive care. This book is aimed at the clinician or scientist who has an interest in renal cell cancer, whether they are academic or nonacademic. The book covers tumor biology, molecular biology, surgery techniques, radiation therapy, personal testimonies, and present and future treatments of the disease that are on the horizon. The goal was to produce a textbook that would act as an authoritative source for scientists and clinicians and interpret the field for trainees in surgery, medicine, radiation oncology, and pathology.

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